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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/784,163	02/24/2004	David Tacha	247232US23	2363

22850 7590 04/27/2009

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EXAMINER	
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ART UNIT	PAPER NUMBER
1641	

NOTIFICATION DATE	DELIVERY MODE
04/27/2009	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/784,163	TACHA, DAVID	
	Examiner	Art Unit	
	JAMES L. GRUN	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 October 2008 and 16 January 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *116-137* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *116-137* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submissions filed on 20 October 2008 and 16 January 2009 have been entered. Claims 116-137 are newly added. Claims 1-115 have been cancelled. Claims 116-137 remain in the case.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to provide an adequate written description of the invention, and failing to adequately teach how to make and/or use the invention, i.e. failing to provide an enabling disclosure.

Claims 116-137 are rejected under 35 U.S.C. 112, first paragraph, for reasons similar to those of record as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. With regard to these claims, the specification, as originally filed, does not provide support for the invention as is now claimed.

Applicant teaches that detection of more than two antigens requires sequential treatments (see e.g.: pages 16-17; original claims 72, 74, 83, 85, 89, or 91) and provides no guidance for simultaneous triple, or more, staining with three, or more, different simultaneous primary and three, or more, different simultaneous secondary antibodies to discern this number of different antigens as is now encompassed by the invention as instantly claimed. Although one of skill in the art might realize from reading the disclosure that simultaneous detection of three different antigens with three simultaneous primary and three simultaneous secondary antibodies is useable in the invention, such possibility of use does not provide explicit or implicit indication to one of skill in the art that such a format was originally contemplated as part of applicant's invention and such possibility of use does not satisfy the written description requirements of 35 U.S.C. § 112, first paragraph. Note that a description which renders obvious a claimed invention is not sufficient to satisfy the written description requirement. Applicant is requested to direct the Examiner's attention to specific passages where support for these newly recited limitations can be found in the specification as filed or is required to delete the new matter.

Applicant teaches performance of a dual staining assay in approximately 2 to 2.5 hours (see e.g. page 16) and a sequential triple staining assay in approximately 2.5 to 3 hours (see e.g. page 17). Although one of skill in the art might realize from reading the disclosure that other times are potentially useable in the invention, such possibility of use does not provide explicit or implicit indication to one of skill in the art that the time ranges as are now claimed, and in particular a time **less than** 2 hours as is now claimed, were originally contemplated as part of applicant's invention and such possibility of use does not satisfy the written description requirements of 35 U.S.C. § 112, first paragraph. Note that a description which renders obvious

a claimed invention is not sufficient to satisfy the written description requirement. Applicant is requested to direct the Examiner's attention to specific passages where support for these newly recited limitations can be found in the specification as filed or is required to delete the new matter.

Moreover, these claims are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicant does not disclose other than the simultaneous use of two secondary antibodies labeled with horseradish peroxidase and alkaline phosphatase. Absent further written description and guidance from applicant, one would not readily envision how to accomplish simultaneous labeling to discern different antigens with different secondary antibodies for the number of primary antibodies and antigens encompassed by the instant claims. The art would also suggest that more than three simultaneous stainings would be difficult to discriminate (see e.g. van der Loos (1999), page 64). A patent is granted for a completed invention, not the general suggestion of an idea and how that idea might be developed into the claimed invention. In the decision of *Genentech Inc. v. Novo Nordisk*, 42 USPQ 2d 1001 (CAFC 1997), the court held that: "[p]atent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable" and that "[t]ossing out the mere germ of an idea does not constitute enabling disclosure." The court further stated that: "when there is no disclosure of any specific starting material or of any of the conditions under which a process is to be carried out, undue experimentation is required; there is a failure to meet the enablement requirements that cannot be rectified by asserting that all the disclosure related to

the process is within the skill of the art", "[i]t is the specification, not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement."

Applicant's arguments filed 20 October 2008 and 16 January 2009 and the declaration of David Tacha, Ph.D., under 37 CFR § 1.132, filed 20 October 2008 have been fully considered but they are not deemed to be persuasive.

Notwithstanding applicant's assertions to the contrary, applicant's amendments have not obviated rejections under this statute for the reasons set forth above.

The declaration of Dr. Tacha and applicant's arguments thereto urge that simultaneous triple and quadruple staining works. This is not found persuasive for the reasons of record because the instant specification teaches only two labels, teaches no functional method as to how to achieve simultaneous staining to discern more than two different antigens bound to the two labels, and teaches that sequential treatments are required for more than two different antigens at a time to use different substrates with the same two enzyme labels to form different colors to discern the different antigens.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 116-137 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 116 and claims dependent thereupon, the interrelationships of the steps of the method are not clear. For example: there is nothing to limit the sample to a tissue or solid phase to provide support for formation of a complex "on" the sample; the interrelationships of the primary and secondary antibodies and the two or more antigens are not clear as to what is specific for what; it is not clear which two antigen-antibody complexes are sufficient for the detecting step, e.g. the detection of a complex of the first secondary antibody bound to a first primary antibody bound to antigen in sample meets the limitation as claimed if the first primary antibody binds antigen in sample and is also antigen for the first secondary antibody; and, it is not clear if the previously performed contacting with a primary antibody cocktail forms part of the method so that it is not clear if this step is to be included in the method for consideration of completion time limitations. The term "stabilize" in these claims is a relative term which renders the claims indefinite. The term "stabilize" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

In claim 119, recitations of "at one third . . . complexes" and "at least one a third . . . complex" are not clear.

In claim 120, recitation of "at one fourth . . . complexes" is not clear.

In claim 124, "the process from application" lacks antecedent basis.

In claim 125 and claims dependent thereupon, the interrelationships of the steps of the method are not clear. For example: it is not clear how a chromogenic reaction comprises of the components as recited; "the process from application" lacks antecedent basis because it is not clear if the previously applied primary antibody cocktail forms part of the method; and, it is not

clear if the previously applied primary antibody cocktail step is to be included in the method for consideration of completion time limitations. The term "stabilize" in these claims is a relative term which renders the claims indefinite. The term "stabilize" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

In claim 126 and claims dependent thereupon, the interrelationships of the steps of the method are not clear. For example: there is nothing to limit the sample to a tissue or solid phase to provide support for formation of a complex "on" the sample; the interrelationships of the primary and secondary antibodies and the two or more antigens are not clear as to what is specific for what; and, it is not clear which two antigen-antibody complexes are sufficient for the detecting step, e.g. the detection of a complex of the first secondary antibody bound to a first primary antibody bound to antigen in sample meets the limitation as claimed if the first primary antibody binds antigen in sample and is also antigen for the first secondary antibody. The term "stabilize" in these claims is a relative term which renders the claims indefinite. The term "stabilize" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

Claims 127, 129, 130, 132, and 133 are entirely vague as to what is being limited because their interrelationships to claim 126 and the interrelationships of the components recited in these claims are not discernible. For example: claim 127 appears duplicative within itself; and, in claims 129 or 130 or 133 the relationships of a third or fourth or fifth or sixth primary antibody cocktail to the previously recited primary antibody cocktail are not clear.

Applicant's arguments filed 20 October 2008 and 16 January 2009 have been fully considered but they are not deemed to be persuasive. Notwithstanding applicant's assertions to the contrary, applicant's amendments have not obviated rejections under this statute for the reasons set forth above.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
> (c) Subject matter developed by another person, which qualifies as prior art only under one or more subsections (e), (f) and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 116-134 are rejected under 35 U.S.C. § 103 (a) as being unpatentable over C. M. van der Loos (1999) in view of Bisgaard et al. (Acta Histo. Cyto. 29: 738, 1996), applicant's admissions, and either of Myers et al. (J. Surg. Pathol. 1: 105, 1995) or Hasui et al. (J. Histochem. Cytochem. 51: 1169, 2003) for reasons similar to those of record in the prior rejection of the similar subject matter of these claims.

The handbook of C. M. van der Loos teaches conventional methods and reagents for immunoenzyme multiple staining protocols. In particular, the reference teaches indirect/indirect simultaneous double staining using cocktails of primary antibodies from different animal species, such as mouse and rabbit (see e.g. pages 14-15, Figs. 3.3 and 3.4), or of different immunoglobulin isotypes (see e.g. page 33, Fig. 3.5) and cocktails of labeled secondary antibodies, such as from a goat, specific for the primary antibodies (see pages 14, 15, 33-36, 45,

and 82-87). Labeling with horseradish peroxidase and alkaline phosphatase is taught, including the use of polymeric conjugates of secondary antibodies and label (see e.g. page 4 or 15). The reference teaches non-commercial and commercial visualization systems and that Diaminobenzidine (DAB) is a chromogen used for immunohistochemical staining when using peroxidase and that Fast Red is a chromogen used for immunohistochemical staining when using alkaline phosphatase (see e.g. pages 45, 91-95). C. M. van der Loos also teaches that indirect/indirect/indirect simultaneous triple staining using cocktails of primary antibodies from different animal species and/or of different immunoglobulin isotypes and/or of different immunoglobulin classes and cocktails of labeled secondary antibodies specific for the primary antibodies (see pages 6, 28, 29, 38, 63-65, and 110-112), as well as other variations such as indirect/indirect/direct simultaneous triple staining, were well known to the art. Combinations of staining protocols to perform staining of more than two antigens in a single sample are also taught, including the use of combinations involving simultaneous double staining with sequential double staining (e.g. page 63). Antigen retrieval is taught. In addition, the conventional use of Tris or phosphate buffers is taught (see e.g. page 77). Although the reference teaches various multiple antigens detected simultaneously (see e.g. pages 16-32 and 103-112), the reference does not teach detection of combinations of antigens as instantly disclosed and does not specifically teach automation of the staining.

Bisgaard et al. teach that water soluble polymer conjugates of secondary antibodies and enzymes, such as horse radish peroxidase (HRP) and calf intestinal alkaline phosphatase (AP), can be used in simultaneous indirect double staining techniques and that such techniques can be performed in less than 2 hours (see page 739). The use of such polymeric conjugates increases

the sensitivity of an assay, can reduce the number of steps in the assay, reduces hands-on time, and reduces the risk of cross-reaction between compounds of the two different staining systems in dual staining techniques.

Either of Myers et al. (1995) or Hasui et al. (2003) teach automation of multiple staining protocols. Myers et al. in particular teach the combination of CD-20 and Ki-67 for simultaneous determination (see e.g. Fig. 2 and page 110). Hasui et al. (2003) also teach elution with buffered hydrochloric acid for immunohistochemical staining of multiple antigens.

Applicant admits that the detection of the combination of P504S, HMWCK and p63 is routine in the art (see page 2).

It would have been obvious to one of ordinary skill in the art at the time the instant invention was made to have selected from the alternatives taught by the handbook of C. M. van der Loos to perform immunoenzyme multiple staining protocols for any desired pair or multiple of antigens, as taught for example in C. M. van der Loos, Myers et al., or applicant's admissions, in view of the direct suggestions in the reference of C. M. van der Loos to do so motivated by the expectation that the conventional reagents and methods would successfully perform as conventionally known in the multiple staining protocols, particularly the polymeric conjugates of Bisgaard et al. suggested for use in C. M. van der Loos known to function in multiple staining protocols performed in less than 2 hours. It would have been obvious to have generated and used monoclonal antibodies in the protocols in order to provide a potentially unlimited source of homogeneous reagent. Automation of multiple staining protocols is well known in the art, as taught in either of Myers et al. (1995) or Hasui et al. (2003), and it would have been obvious to one of ordinary skill in the art at the time the instant invention was made to have automated the

protocols taught by the handbook of C. M. van der Loos, as modified particularly by the teachings of Bisgaard et al., for the benefits of standardization, accuracy, and consistency taught in either of Myers et al. (1995) or Hasui et al. (2003).

Thus, the claimed invention as a whole was clearly prima facie obvious, especially in the absence of evidence to the contrary.

Claims 116-137, particularly 135-137, are rejected under 35 U.S.C. § 103(a) as being unpatentable over C. M. van der Loos (1999) in view of Bisgaard et al. (Acta Histo. Cyto. 29: 738, 1996), applicant's admissions, and either of Myers et al. (J. Surg. Pathol. 1: 105, 1995) or Hasui et al. (J. Histochem. Cytochem. 51: 1169, 2003), as applied to claims 116-134 above, and further in view of Chien et al. (US 6,537,745), Miller et al. (US 5,487,975), and any or all of Loor et al. (US 4,690,890), Philo et al. (US 5,108,896), Diamandis et al. (US 5,089,423), and Damaj et al. (US 2002/0173053).

The teachings of the combination of C. M. van der Loos (1999), Bisgaard et al., applicant's admissions, Myers et al. and/or Hasui et al. are as set forth above. In contrast to the invention as instantly disclosed and/or claimed the references do not teach additional components in a buffer such as bovine serum albumin or preservatives.

Chien et al. teach that substitution of reagents in a buffer with reagents performing essentially the same function is well known in the art, such as substitution of borate with phosphate buffering agents, or substitution of gelatin with albumin or other blocking agents (cols. 3, 6-7, 9), or substitution of a single blocking agent with a mixture thereof (see cols. 6-7),

or substitution of TWEEN-20 for other detergents, or substitution of sodium azide with other anti-bacterial agents.

Miller et al. teach that a diluent for immunohistochemical staining can contain a detergent and a preservative which do not interfere with the immunochemical reaction (see e.g. cols. 2-3). The reference teaches that sodium azide should be avoided if peroxidase staining reagents are to be used. An exemplary preservative well known to the art to not interfere with immunochemical reactions includes 2-methyl-4-isothiazolin-3-one (see e.g. col. 3). Gamma globulin and casein are included in the diluent for blocking non-specific interactions of immunochemical components with tissue sections (see e.g. cols. 3-4).

Loor et al. teach immunoassays, for the detection of multiple antigens, which may use different enzyme labels on antigen-specific antibodies in a mixture to detect the different antigens (col. 6-8). The reference teaches that immunoassays are typically performed at pH 6-9 (col. 8). The reference teaches antibody diluents having 0.05M Tris, 0.05% preservative, 1.0% bovine serum albumin (BSA), and 100 mM NaCl (col. 15), or 0.05M Tris, 0.05% preservative, 5.0% BSA, and 100 mM NaCl (col. 16).

Philo et al. teach immunoassays, for the detection of multiple antigens, which may use different labels on antigen-specific antibodies in a mixture to simultaneously detect the different antigens. The antibody mixtures were diluted in a buffer comprising 0.1M Tris/HCl, 0.2% sodium azide, 0.5% BSA, serum, and 100 mM NaCl (col. 11).

Diamandis et al. teach antibody diluents of various formulations, e.g.: 0.01M Tris, 0.01% sodium azide, 1% BSA, and 0.01% thimerosal (col. 11); 0.05M Tris, 0.05% sodium azide, 0.5%

BSA, 0.01% TWEEN, 150 mM NaCl (col. 14); or, 0.05M Tris, 0.05% sodium azide, 1.0% BSA, 150 mM NaCl (col. 20).

Damaj et al. teach simultaneous antigen detection by immunohistochemistry. Mixtures of different antibody populations, specific for different markers to be determined, each conjugated to a different enzyme, e.g. horseradish peroxidase and alkaline phosphatase (see [0027], [0036], [0046] and claim 7), were made in blocking buffer (see [0027]). Blocking buffer comprised borate buffer, 0.05% TWEEN 20, 0.25% bovine serum albumin, and 0.05% sodium azide. Substrates for the different enzymes were added sequentially ([0047]). The reference teaches alternative labeling known to the art ([0006]). The reagents are provided in a kit ([0013]).

It would have been obvious to one of ordinary skill in the art at the time the instant invention was made to have used buffers including preservatives and carrier/blocker proteins in the compositions and methods of van der Loos, as modified, in order to achieve a desired pH in the range of 6-9, preserve the composition, and prevent non-specific loss of the active composition components because to do so is conventional in the immunoassay art as taught in any or all of Miller et al., Loor et al., Philo et al. (US 5,108,896), Diamandis et al. (US 5,089,423), and Damaj et al. (US 2002/0173053). One would have been motivated to have adjusted the buffer components within the ranges known to the art, such as in the compositions taught in any of the references, with an expectation that the components would perform their desired functions, such as buffering, blocking, or preserving, in the antibody diluent/blocking compositions. It would have also been obvious to one of ordinary skill in the art at the time the instant invention was made to have substituted reagents in the antibody buffer compositions for

use in the methods of van der Loos, as modified, with those performing essentially the same function because to do so is routine in the art as taught in Chien et al. In particular, it would have been obvious to have included a preservative other than sodium azide, such as one containing 2-methyl-4-isothizolin-3-one as taught in Miller et al., for use with peroxidase staining reagents in the compositions and methods of van der Loos, as modified, in view of the direct suggestion in Miller et al. of the desirability to include such a preservative when staining with peroxidase reagents.

Thus, the claimed invention as a whole was clearly prima facie obvious, especially in the absence of evidence to the contrary.

Claims 116-120, 122-130, and 132-134 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Mason et al. (J. Can. Res. Clin. Oncol. 101: 13, 1981) in view of applicant's admissions, and any or all of Bisgaard et al. (Acta Histo. Cyto. 29: 738, 1996), Ward et al. (US 4,687,732), Ohbayashi et al. (US 6,252,053), and Shi et al. (Appl. Immunohistochem. Mol. Morph. 7: 201, 1999).

Mason et al. teach simultaneous double immunoenzymatic labeling using cocktails of primary antibodies from different animal species and cocktails of labeled secondary antibodies specific for the primary antibodies (see e.g. Figs. 1 and 7). Labeling with horseradish peroxidase and alkaline phosphatase is taught. Simultaneous detection of κ and λ chains is specifically exemplified (see e.g. Figs. 2 or 3). Mason et al. suggest conjugates of enzymes with secondary antibodies as a substitute for the enzyme-anti-enzyme complexes used as labels in the

exemplified methods (see page 20). In contrast to the invention as instantly claimed, the reference does not teach coupling secondary antibodies to polyenzyme moieties.

Applicant admits that Diaminobenzidine (DAB) is a widely used chromogen for immunohistochemical staining when using peroxidase and that Fast Red is a widely used chromogen for immunohistochemical staining when using alkaline phosphatase (see e.g. page 14).

Bisgaard et al. teach that water soluble polymer conjugates of secondary antibodies and enzymes, such as horse radish peroxidase (HRP) and calf intestinal alkaline phosphatase (AP), can be used in simultaneous indirect double staining techniques and that such techniques can be performed in less than 2 hours (see page 739). The reference teaches the polymers as a substitute for other labels such as streptavidin-biotin multi-step layering. The use of such polymeric conjugates increases the sensitivity of an assay, can reduce the number of steps in the assay, reduces hands-on time, and reduces the risk of cross-reaction between compounds of the two different staining systems in dual staining techniques.

Ward et al. teach polymerized enzyme-antibody conjugates for immunohistochemistry as a substitute for other labels such as peroxidase-anti-peroxidase complexes, or avidin-biotin multi-step layering.

Ohbayashi et al. teach enzyme-antibody polymer conjugates for immunohistochemistry as a substitute for other labels such as streptavidin-biotin multi-step layering.

Shi et al. teach polymerized enzyme-antibody conjugates for immunohistochemistry as a substitute for other labels such as peroxidase-anti-peroxidase complexes.

It would have been obvious to one of ordinary skill in the art at the time the instant invention was made to have substituted the conjugates of any of Bisgaard et al., Ward et al., Ohbayashi et al., or Shi et al. for the enzyme-anti-enzyme complexes used in Mason et al. in view of the direct suggestions of all of Mason et al., Ward et al., and Shi et al. to make the substitution. It would have been obvious to have generated and used monoclonal antibodies in the protocols in order to provide a potentially unlimited source of homogeneous reagent. It would have been further obvious to one of ordinary skill in the art at the time the instant invention was made to have substituted widely known and used chromogens, as admitted by applicant, in the simultaneous double immunoenzymatic labeling of Mason et al., as modified, in view of their notoriously well known use in immunoenzymatic labeling with peroxidase and alkaline phosphatase. One would have had an extremely reasonable expectation that the conventional reagents and methods would successfully perform as conventionally known in the multiple staining protocols, particularly the polymeric conjugates of Bisgaard et al. known to function in multiple staining protocols performed in less than 2 hours.

Thus, the claimed invention as a whole was clearly prima facie obvious, especially in the absence of evidence to the contrary.

Applicant's arguments filed 20 October 2008 and 16 January 2009 and the declaration of David Tacha, Ph.D., under 37 CFR § 1.132, filed 20 October 2008 have been fully considered but they are not deemed to be persuasive.

Applicant's arguments with respect to the claims have been considered but are moot in view of the new ground(s) of rejection. However, applicant's arguments, as related to the new

grounds of rejection set forth in this Office action necessitated by applicant's amendments, have been considered below.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Moreover, the exercise of pairing the teachings of only some of the cited references, as argued by applicant, is equally unpersuasive. The question is not whether the combination of part or all of the references cited by the examiner was obvious to the applicant, but whether the combination was obvious to a person of ordinary skill in the art.

The declaration of Dr. Tacha and applicant's arguments thereto urge that dextran polymers comprising secondary antibodies and multiple enzyme copies are different from the poly enzyme conjugates in the claims. These are not found persuasive because the allegation of a difference and arguments thereto are not supported by any limitations found in the specification or in the instant claims. Notwithstanding applicant's assertions to the contrary: the reference of Shi et al., noted at page 12 of the specification, is considered merely exemplary of poly enzyme conjugates because the reference provides no support for a poly alkaline phosphatase conjugate, as clearly admitted in the declaration of Dr. Tacha (see ¶ 12); and, the reference of Shi et al. clearly teaches the "EnVision" reagent as exemplary of polymeric amplification (see e.g. page 202 and Table 5) and thus provides description of this reagent for this use. Further, although claims are interpreted in light of the disclosure, limitations from the specification are not imported into the claims unnecessarily. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057

(Fed. Cir. 1993). See also *In re Zletz*, 893 F.2d 319, 321-22, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989) ("During patent examination the pending claims must be interpreted as broadly as their terms reasonably allow.... The reason is simply that during patent prosecution when claims can be amended, ambiguities should be recognized, scope and breadth of language explored, and clarification imposed.... An essential purpose of patent examination is to fashion claims that are precise, clear, correct, and unambiguous. Only in this way can uncertainties of claim scope be removed, as much as possible, during the administrative process."). Moreover, for the reasons of record and as set forth above, polymeric conjugates of secondary antibodies with enzymes or other markers were well known to the art as taught by any or all of Bisgaard et al. (Acta Histo. Cyto. 29: 738, 1996), C. M. van der Loos (1999), Ward et al. (US 4,687,732), Ohbayashi et al. (US 6,252,053), and Shi et al. (Appl. Immunohistochem. Mol. Morph. 7: 201, 1999). Moreover, Shi et al. (US 2003/0017491) teach that polymerized enzyme-secondary antibody conjugates, such as those of Ohbayashi et al. (i.e. "Nichirei") or Shi et al. (1999; i.e. "ImmunoVision"), were known to the art and commercially available prior to 2003 (see [0112]).

Notwithstanding the declaration of Dr. Tacha and applicant's arguments thereto, simultaneous immunohistochemical multiple staining was well known to the art, including to DAKO, as evidenced by any of C. M. van der Loos (1999), Bisgaard et al., or Mason et al. Indeed, the excerpts from the DAKO handbook attached to applicant's declaration clearly teach indirect simultaneous staining with time saving antibody cocktails (see e.g. pages 63-64).

Notwithstanding the declaration of Dr. Tacha and applicant's arguments thereto, the benefits of using polymeric conjugates in simultaneous indirect multiple staining techniques in increasing the sensitivity of an assay, reducing the number of steps in the assay, reducing hands-

on time, and reducing the risk of cross-reaction between compounds of the two different staining systems in dual staining techniques was clearly appreciated by the art, especially by DAKO, as evidenced by Bisgaard et al., a reference also known to and cited by C. M. van der Loos (1999).

The declaration of Dr. Tacha and applicant's arguments thereto urge that simultaneous triple and quadruple staining works. This is not found persuasive for the reasons of record because the instant specification teaches only two labels, teaches no functional method as to how to achieve simultaneous staining to discern more than two different antigens bound to the two labels, and teaches that sequential treatments are required for more than two antigens at a time to use different substrates with the same two enzymes to form different colors.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Sabattini et al. (J. Clin. Pathol. 51: 506, 1998) teach water soluble polymer conjugates of secondary antibodies and horse radish peroxidase (HRP) for use in indirect immunohistochemical staining techniques.

Shi et al. (US 2003/0017491) teach that polymerized enzyme-secondary antibody conjugates were known to the art and commercially available prior to 2003 (see [0112]).

Ferri et al. (J. Histochem. Cytochem. 45: 155, 1997) teach quadruple immunofluorescence with 4 primary antibodies from different animal species labeled indirectly with labeled secondary antibodies specific for the primary antibodies.

Nakane (J. Histochem. Cytochem. 16: 557, 1968) teaches elution with buffered or unbuffered hydrochloric acid for immunohistochemical staining of multiple antigens.

Krajewski et al. (Anal. Biochem. 236: 221, 1996) teach an antibody diluent for detection of multiple antigens comprising 0.01M Tris, 2% BSA, 0.1% serum, 5% skim milk, 0.05-0.1% TWEEN 20, 0.01% sodium azide or 0.01% thimerosal, and 150 mM NaCl. The reference teaches a formulation of phosphate-buffered saline having 41.5 mM phosphate (K_2HPO_4/NaH_2PO_4) and 120 mM NaCl.

Boscato et al. (Clin Chem. 32 : 1491, 1986) teach a phosphate-buffered saline having 10 mM phosphate (KH_2PO_4/Na_2HPO_4), 0.1% sodium azide, 10 mM EDTA, and 150 mM NaCl, also containing 0.1 % serum albumin for dilution of antibody.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to James L. Grun, Ph.D., whose telephone number is (571) 272-0821. The examiner can normally be reached on weekdays from 9 a.m. to 5 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mark Shibuya, SPE, can be contacted at (571) 272-0806.

The phone number for official facsimile transmitted communications to TC 1600, Group 1640, is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application, or requests to supply missing elements from Office communications, should be directed to the Group receptionist whose telephone number is (571) 272-1600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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